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(57) Abstract

Methods and compositions for attenuating or mitigating; suppressing; preventing; delaying onset of; or, reversing exogenous protein inhibitor syndromes, exemplified by clotting factor (e.g., Factor VIII) inhibitor syndromes. The described methods use a CD40:CD154 binding interruptor, such as CD154 blocking agent, to attenuate or ameliorate counter–adaptive, bioinhibitory humoral immunity directed against an exogenous protein of therapeutic value.

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CD154 BLOCKADE THERAPY FOR THERAPEUTIC PROTEIN INHIBITOR SYNDROME

Related Applications

This is a continuation-in-part of prior U.S. Provisional S.N. 60/050,276, filed June 20, 1997. The teachings of the earlier-filed Provisional patent application are incorporated herein by reference.

Field of the Invention

The invention relates generally to the suppression of unwanted immune responses, particularly of counter-adaptive T-lymphocyte mediated immune responses. The invention relates in particular to the prevention, treatment, suppression and reversal of immunological inhibition of the therapeutic activity of exogenously-administered proteins or other biological therapeutic agents.

Background of the Invention

Hemophilia A is an X-linked genetic deficiency disease that affects one to two males in every 10,000 live births. Individuals with hemophilia A have a partial or complete functional deficiency of endogenous clotting Factor VIII (FVIII), and must receive purified or recombinant FVIII replacement therapy. Lusher et al. (1993), 328 N. Engl. J. Med. 453-459. Approximately 15% of individuals with hemophilia A develop high-titer antibody responses (i.e., > 10 BU/mL) to their FVIII replacement therapeutic. These individuals are referred to as "high responders." McMillan et al. (1988), 71 Blood 344-348. Such antibodies, called FVIII inhibitors, block the function (bioactivity) of the replacement FVIII therapeutic agent, by binding to the administered, exogenous FVIII. This counter-adaptive, humoral immune response makes the treatment of bleeding events in high responders problematic. Minor bleeding episodes (e.g., hemarthroses) are often successfully treated with activated prothrombin complex concentrates (e.g., Autoplex or

FEIBA, FVIII inhibitor bypass activity), but severe bleeding is difficult to control with these agents. Brettler (1996), 9 Clin. Hematol. 319-329. The majority of high responders have low levels of the FVIII inhibitor antibodies until they receive a subsequent infusion of FVIII, which stimulates (boosts) the production of the blocking antibodies. Therefore, the re-induction of inhibitory antibodies in high responder individuals is highly predictable. Brettler (1996), 9 Clin. Hematol. 319-329. Prophylaxis with FVIII has been shown to reduce the incidence of intraarticular hemorrhage and chronic arthropathy in hemophiliacs. Liesner et al. (1996), 92 Br. J. Haematol. 973-978. However, because of the FVIII specific humoral immune response in high responders, such individuals cannot receive prophylactic therapy with FVIII.

High responders with an inducible, vigorous immune response to FVIII are often treated with regular infusions of very high doses of FVIII in regimens designed to induce "tolerance" to the exogenous FVIII therapeutic. Brettler (1996), 9 Clin. Hematol. 319-329. In some cases, immunomodulator agents (glucocorticoids, cyclophosphamide, intravenous immunoglobulin (IVIg)) are added to the tolerance regimens. Tolerance therapy is effective in 50% to 80% of high responder individuals. Mariani et al. (1994), 72 Thromb. Haemostasis 155-158. The cost of such therapy ranges from $200,000US to nearly $1 million US per individual. Because infusion of FVIII induces very high levels of inhibitors in high responders, during the tolerance induction period – which often lasts from three to eight months, these individuals cannot be treated with FVIII if they do have a bleed. Brettler (1996), 9 Clin. Hematol. 319-329.

Following successful tolerance induction, individuals are often maintained on prophylactic FVIII infusions twice to three times weekly. Brettler (1996), 9 Clin. Hematol. 319-329. The mechanism of tolerance induction using these protocols is unclear, but may involve the induction of anti-idiotypic antibodies (Gilles et al. (1996), 97 J. Clin. Invest. 1382-1388) or more direct suppression of the B cell clones making the FVIII inhibitor antibodies. In addition, IVIg preparations contain anti-idiotypic antibodies to FVIII inhibitors, which may explain the efficacy of this therapy in some high responders. Sultan

et al. (1991), 91 Am. J. Med. 5A-35S-5A-39S. In very severe and life-threatening cases that necessitate the use of FVIII therapy for bleeding, FVIII inhibitors can be removed by extracorporeal immunoabsorption on anti-Ig or Protein A columns. Knobl et al. (1995), 74 Thromb. Haemostasis 1035-1038; Gjorstrup et al. (1991), 61 Vox Sang 244-250. These protocols are time-consuming and result in 50% to 75% reduction in total serum immunoglobulin (Ig) levels (Knobl et al. (1995), 74 Thromb. Haemostasis 1035-1038) thus potentially increasing the risk of infection.

Similar complications of protein replacement therapy have been encountered in treatment of other congenital or acquired protein deficiency diseases, including deficiencies of other clotting factors, blood or plasma proteins, growth factors, and the like. Furthermore, analogous complications have been encountered in other clinical settings, such as where a recombinantly produced counterpart of an endogenous, but rare or sequestered protein is administered therapeutically. For example, analogous complications have been encountered in therapies involving the administration of recombinant cytokines, lymphokines, growth factors or enzymes. One example is the administration of erythropoeitin (EPO) for treatment of anemia. Another is the administration of interferon β (IFN β) for treatment of multiple sclerosis (MS). Still another is the administration of human growth hormone (hGH) for treatment to accellerate growth. Analogous complications also have been encountered where a microbial protein is administered therapeutically, such as where streptokinase is administered for treatment of stroke or another type of vascular occlusion.

There is accordingly a need for improved or more effective immunosuppressive or immunomodulatory treatments for minimizing or suppressing the development of inhibitory antibodies that bind to, and block the therapeutic activity of, exogenously administered proteins. In particular, there is a need for treatments that do not require pan-T cell immunosuppression, i.e., treatments that do not leave the recipient vulnerable to malignancies or opportunistic infection. More pointedly, there is a need for reversing or

suppressing inhibitor syndromes that preclude the administration of a needed protein therapeutic, such as FVIII, to individuals in need thereof.

Summary of the Invention

It is an object of this invention to provide an immunomodulatory agent that mitigates counter-adaptive T cell responses without the need for pan-T cell immunosuppression. Another object is to provide an immunomodulatory agent that mitigates severity of a counter-adaptive humoral immune response to a needed, exogenous protein therapeutic. Another object is to provide an immunomodulatory agent that delays onset of a counter-adaptive humoral immune response to a needed, exogenous protein therapeutic. Another object is to provide an immunomodulatory agent that suppresses or reverses a counter-adaptive humoral immune response to a needed, exogenous protein therapeutic. A further object is to provide an immunomodulatory agent that interrupts delivery of a costimulatory signal to activated T cells, particularly a costimulatory signal for immunoglobulin production. A particular object is to provide a CD40:CD154 binding interruptor, such as a CD154 blocking agent, for use in therapy, particularly for use in therapy to mitigate, delay onset of, or reverse a counter-adaptive inhibitory antibody response to a needed, exogenous protein therapeutic agent, such as FVIII.

The present invention rests on the discovery that use of a CD40:CD154 binding interruptor, such as a CD154 blocking agent, attenuates, mitigates, suppresses, prevents, delays, inhibits or reverses counter-adaptive inhibitory antibody responses to protein antigens, *without* the need for pan-suppression of the recipient's immune system. More precisely, the present invention rests on the discovery that use of a CD154 blocking agent attenuates, mitigates, suppresses, prevents, delays, inhibits or reverses undesirable inhibitory humoral immunity that blocks bioactivity of a protein therapeutic administered to an individual to replace or augment native bioactivity of an endogenous, but defective protein, such as a clotting factor, e.g., FVIII.

The invention accordingly provides methods and compositions for immunomodulatory therapy for exogenous protein inhibitor syndromes. A first method

attenuates or mitigates severity of an exogenous protein inhibitor syndrome. A second method suppresses adverse effects of the syndrome. A third method prevents the development of the syndrome. A fourth method delays onset of the syndrome. A fifth method inhibits development of the syndrome. A sixth method reverses the syndrome. A seventh method preserves therapeutic efficacy of an exogenous protein, such as a therapeutic protein administered to replace or supplement a native, but defective protein. An eighth method restores therapeutic efficacy of such an exogenous protein. All of the foregoing methods involve treating a subject afflicted with, or at risk of developing, an exogenous protein inhibitor syndrome, by which is meant a counter-adaptive humoral immune response that blocks (interferes with) bioactivity of the exogenous protein, with a CD40:CD154 binding interruptor, by which is meant any agent that interrupts the binding of CD40 Ligand (i.e., CD40L, also known as CD154 or the 5c8 antigen, and sometimes referred to in the art as gp39) to its counter or cognate receptor (here, CD40). Preferably, the binding interruptor is a CD154 (CD40L) blocking agent, by which is meant any agent that binds to CD154 and prevents or interferes with its binding to counter receptors (e.g., CD40). An exemplary CD154 blocking agent is a monoclonal antibody (MAb), particularly one having the antigen-specific binding characteristics of the 5c8 MAb disclosed in U.S. Patent 5,474,771, the teachings of which are incorporated herein by reference.

As mentioned above, the present invention can be practiced to attenuate or ameliorate inhibitor syndromes directed against exogenous proteins that are administered to replace or augment the bioactivity of a native (endogenous) protein that is defective. The invention also can be practiced to attenuate or ameliorate inhibitor syndromes directed against other exogenous proteins, including any exogenous protein that is administered for therapeutic purposes. For example, the invention can be practiced to suppress, reverse or inhibit an inhibitor response directed against any recombinantly-produced protein therapeutic, particularly a protein therapeutic having a primary structure (sequence) substantially similar to (e.g., substantially identical to) a native, functional, but rare protein, or a native protein that naturally is sequestered in a particular body structure or

compartment, such as bone marrow, a lymph node, or the central nervous system. Similarly, the the invention can be practiced to suppress, reverse or inhibit an inhibitor response to a recombinant version of a native protein that is transiently expressed, or expressed only in response to specific environmental stimuli or at specific points during development. Thus, the invention can be practiced to attenuate or ameliorate an inhibitor response against a growth hormone, wound healing factor (e.g., a tissue regeneration factor or differentiation factor), cytokine or lymphokine (e.g., a colony stimulating factor, stem cell factor, interferon, or interleukin), enzyme (e.g., glucocerebrosidase), blood clotting factor (e.g., thrombin, prothrombin, Factor V, Factor VII, Factor VIII, Factor IX, Factor X, Factor XI, or Factor XII), or other plasma component (e.g., albumin, tissue plasminogen activator). Further, the invention can be practiced to attenuate or ameliorate an inhibitor response against a foreign protein, particularly a bacterial protein (e.g., streptokinase) that is administered for therapeutic purposes (e.g., treatment of vascular occlusion). Preferred subjects on whom the invention is practiced are human subjects. In particular, the invention can be practiced to attenuate or ameliorate clotting factor inhibitor syndromes in hemophiliacs.

The foregoing and other objects, features and advantages of the present invention, as well as the invention itself, will be more fully understood from the following description of preferred embodiments.

Detailed Description of the Invention

T cell activation, and immunological processes dependent thereon, requires both T cell receptor (TCR) mediated signals and simultaneously delivered costimulatory signals. An important costimulatory signal is delivered by the ligation of CD40 on an antigen-presenting cell, such as a B cell, by CD40L (CD154) on a T cell. Human CD40 is a 50 kD cell surface protein expressed on mature B cells, as well as on macrophages and activated endothelial cells. CD40 belongs to a class of receptors involved in programmed cell death, including Fas/CD95 and the tumor necrosis factor (TNF) alpha receptor. Human CD154 (CD40L) is a 32 kD type II membrane glycoprotein with homology to TNF alpha that is

transiently expressed, transiently, primarily on activated T cells. CD40:CD154 binding has been shown to be required for all T cell-dependent antibody responses. In particular, CD40:CD154 binding provides anti-apoptotic and/or lymphokine stimulatory signals.

The importance of CD40:CD154 binding in promoting T cell dependent biological responses was more fully appreciated when it was discovered that X-linked hyper-IgM syndrome (X-HIGM) in humans is the phenotype resulting from genetic lack of functional CD154. Affected individuals have normal or high IgM levels, but fail to produce IgG, IgA or IgE antibodies, and suffer from recurrent, sometimes severe, bacterial and parasitic infections, as well as an increased incidence of lymphomas and abdominal cancers. A similar phenotype is observed in non-human animals rendered nullizygous for the gene encoding CD154 (knockout animals). B cells of CD154 nullizygotes can produce IgM in the absence of CD40L:CD154 binding, but are unable to undergo isotype switching, or to survive normally after affinity maturation. Histologically, lymph node germinal centers fail to develop properly, and memory B cells are absent or poorly developed. Functionally, these defects contribute to a severe reduction or absence of a secondary (mature) antibody response. Defects in cellular immunity are also observed, manifested by an increased incidence of bacterial and parasitic infections. Many of these cell-mediated defects are reversible by administration of IL-12 or IFN-gamma. These observations substantiate the view that normal CD40:CD154 binding promotes the development of Type I T-helper cell immunological responses.

Blockade of the CD40:CD154 interaction during immunization with protein antigens can specifically block the antibody response to that antigen in mice. Foy et al. (1993), 178 J. Exp. Med. 1567-1575. For example, anti-CD154 antibodies can block the induction of anti-collagen antibodies in collagen-induced arthritis. Durie et al. (1993), 261 Science 1328-1330. Anti-CD154 antibodies can reduce anti-dsDNA and anti-nucleosomal autoantibodies in mice with spontaneous lupus. Mohan et al. (1995), 154 J. Immunol. 1470-1480. In addition, anti-CD154 antibodies can reduce symptoms in mice with experimental autoimmune encephalomyelitis (EAE), a model of MS. Similar results have

been reported in rodent models of graft-versus-host-disease, mercuric chloride induced glomerulonephritis, and inflammatory bowel disease.

CD40:CD154 blockade thus may provide potentially powerful therapies for attenuating or ameliorating unwanted humoral immune responses, particularly in the context of autoimmune diseases or, indeed, wherever the target antigen is a protein of therapeutic value, which value is impeded by a counter-adaptive immune response. However, despite numerous reports of promising results, studies performed in rodent models of induced counter-adaptive immunological disease (e.g., autoimmunity) have correlated poorly with the outcome of testing in actual disease contexts, or even in larger animal preclinical model systems (e.g., primates).

Disclosed herein are protocols for assessing the effects of a preferred CD154 blocking agent, a humanized MAb having the antigen-specific binding properties of MAb 5c8 (Lederman et al., J. Exp. Med. 175:1091-1101,1992), in preclinical models believed predictive of therapeutic efficacy in treatment of exogenous protein inhibitor syndromes. Specifically, the present models involve CD154 blockade therapy to attenuate or ameliorate bioinhibitory humoral immunity specific for clotting factors (e.g., FVIII) and lymphokines (e.g., IFN β). These models can be adapted, through no more than routine manipulation, for use to establish efficacy of CD154 blockade therapy to attenuate or ameliorate inhibitory humoral immunity directed against any protein of therapeutic value.

The following discussion illustrates and exemplifies the variety of contexts and circumstances in which the invention can be practiced, as well as providing proof-of-principle studies involving specific embodiments of the invention.

Subjects for Treatment

The invention can be used for treatment or prophylaxis of any mammalian subject in need of, or already receiving, protein replacement therapy, indeed any protein therapeutic. Subjects accordingly are afflicted with, or at risk of, developing exogenous protein inhibitor syndrome. For example, hemophiliacs being treated with exogenous

FVIII are at substantial risk of becoming "high responders," whereafter FVIII loses effectiveness for its intended purpose of suppressing bleeding events. Accordingly, the invention is particularly suitable for use with hemophiliacs. Procedures for determining whether a hemophiliac has developed an inhibitory response against therapeutically administered FVIII, and/or has become a high responder, are well known. See, e.g., Hematology: Clinical and Laboratory Practice, vol. 2, Bick, ed., Mosby-Year Book, Inc., publ. (1993), pp.1544-1548. Preferably, the subject mammal is a primate, more preferably a higher primate, most preferably a human. In other embodiments, the subject may be another mammal afflicted with, or at risk of, developing an exogenous protein inhibitor syndrome, particularly a mammal of commercial importance, or a companion animal or other animal of value, such as a member of an endangered species. Thus, subjects also include, but are not limited to, sheep, horses, cattle, goats, pigs, dogs, cats, rabbits, guinea pigs, hamsters, gerbils, rats and mice.

Exemplary CD40:CD154 Binding Interruptors

Therapeutic compounds useful for practice of the invention include any compound that blocks the interaction of cell surface CD40 (e.g., on B cells) with CD40L (CD154) expressed, e.g., on the surface of activated T cells. CD40:CD154 binding interruptor compounds, such as CD154 blocking agents, that are specifically contemplated include polyclonal antibodies and monoclonal antibodies (MAbs), as well as antibody derivatives such as chimeric molecules, humanized molecules, molecules with reduced effector functions, bispecific molecules, and conjugates of antibodies. In a preferred embodiment, the antibody has substantially the same antigen-specific binding characteristics as MAb 5c8, as described in U.S. Patent 5,474,771, the disclosure of which is hereby incorporated by reference. In a currently highly preferred embodiment, the antibody is a humanized 5c8 (hu5c8). Other known antibodies against CD154 include antibodies ImxM90, ImxM91 and ImxM92 (disclosed by Immunex Corp., Seattle WA), an anti-CD40L MAb commercially available from Ancell (clone 24-31, catalog # 353-020, Bayport, MN), and an anti-CD154 MAb commercially available from Genzyme (Cambridge, MA, catalog #

80-3703-01). Also commercially available is an anti-CD154 MAb from PharMingen (San Diego, catalog #33580D). Numerous additional anti-CD154 antibodies have been produced and characterized (see, e.g., WO 96/23071 of Bristol-Myers Squibb, the specification of which is hereby incorporated by reference).

The invention also includes use of CD154 blocking agents that are derived from, or engineered from the above-mentioned and equivalent MAbs, such as complete Fab fragments, $F(ab')_2$ compounds, V_H regions, F_V regions, single chain antibodies (see, e.g., WO 96/23071), polypeptides, fusion constructs of polypeptides, fusions of CD40 (such as CD40Ig, as in Hollenbaugh et al., J. Immunol. Meth. 188:1-7, 1995, which is hereby incorporated by reference), and small molecule compounds such as small semi-peptidic compounds or non-peptide compounds, all capable of blocking or interrupting CD40:CD154 binding. Procedures for designing, screening and optimizing small molecules are provided in PCT/US96/10664, filed June 21, 1996, the specification of which is hereby incorporated by reference.

Thus, the invention can be practiced with MAb-derived, CD154 blocking agents created using standard recombinant DNA techniques (Winter and Milstein, Nature 349: 293-99, 1991). One class of such CD154 blocking agents includes chimeric antibodies, or fusion proteins constructed by joining nucleic acid encoding the antigen binding domain of a non-human mammalian antibody (e.g., a mouse or rat antibody) of desired specificity to nucleic acid encoding a human immunoglobulin (Ig) constant region. Cabilly et al., United States Pat. No. 4,816,567; Morrison et al., Proc. Natl. Acad. Sci. 81: 6851-55, 1984. Chimeric antibody polypeptides expressed from such constructs generally have lower immunogenicity, when used for human therapy or prophylaxis, than the non-human antibody from which the chimera was derived. A second class of such CD154 blocking agents includes recombinant "humanized" or "primatized"antibodies. Humanized or primatized antibodies are antibodies are genetically engineered from non-human mammalian antibodies having the desired specificity, by replacing some or all of the codons for amino acids not required for antigen binding with codons for amino acids from

corresponding regions of a human or primate Ig light or heavy chain gene. That is, they are chimeras comprising mostly human immunoglobulin sequences into which the regions responsible for antigen specific binding have been genetically inserted (see, e.g., PCT patent application WO 94/04679). Humanized antibodies generally have even lower immunogenicity in vivo than chimeric antibodies. Currently, a humanized MAb having substantially the same antigen specificity as MAb 5c8 (herein, hu5c8) is preferred for practice of the invention.

Another class of MAb-derived CD154 blocking agents useful in the invention includes human antibodies, which can be produced in transgenic nonhuman mammals, into whom one or more human immunoglobulin transgenes have been integrated. Such animals may be used as a source for splenocytes for producing human hybridomas, as described in U.S. 5,569,825.

Of course, any antigen-specific binding fragment of one of the foregoing MAbs or MAb derived therapeutic agent can be used in the present invention, provided that the fragment is sufficiently large to sterically impede CD154 binding to its counter-receptor. Thus, MAb fragments and univalent MAbs can be used. Univalent antibodies comprise a heavy chain/light chain dimer bound to the Fc (or stem) region of a second heavy chain. "Fab region" refers to those portions of the chains which are roughly equivalent, or analogous, to the sequences which comprise the Y branch portions of the heavy chain and to the light chain in its entirety, and which collectively (in aggregates) have been shown to exhibit antibody activity. A Fab protein includes aggregates of one heavy and one light chain (commonly known as Fab'), as well as tetramers which correspond to the two branch segments of the antibody Y, (commonly known as $F(ab)_2$), whether any of the above are covalently or non-covalently aggregated, so long as the aggregation is capable of selectively reacting with a particular antigen or antigen family.

In addition, standard recombinant DNA techniques can be used to alter the binding affinities of recombinant antibodies with their antigens by altering amino acid residues in the vicinity of the antigen binding sites. The antigen binding affinity of a humanized

antibody may be increased by mutagenesis based on molecular modeling (Queen et al., Proc. Natl. Acad. Sci. 86:10029-33, 1989; PCT patent application WO 94/04679). It may be desirable to increase or to decrease the affinity of the antibodies for CD154, depending on the targeted tissue type or the particular treatment schedule envisioned. This may be done utilizing phage display technology (see, e.g., Winter et al., Ann. Rev. Immunol. 12:433-455, 1994; and Schier et al., J. Mol. Biol. 255:28-43, 1996, which are hereby incorporated by reference). For example, it may be advantageous to treat a patient with constant levels of antibodies with reduced affinity for CD154 for semi-prophylactic treatments. Likewise, antibodies with increased affinity for CD154 may be advantageous for short-term treatments.

Routes of Administration

The CD40:CD154 binding interruptors, including CD154 blocking agents, used in the invention can be administered in any manner which is medically acceptable. Depending on the specific circumstances, local or systemic administration may be desirable. Preferably, the agent is administered via a parenteral route such as by an intravenous, intraarterial, subcutaneous, intramuscular, intraorbital, intraventricular, intraperitoneal, subcapsular, intracranial, intraspinal, or intranasal injection, infusion or inhalation. The agent also can be administered by implantation of an infusion pump, or a biocompatible or bioerodable sustained release implant, into the recipient host, either before or after implantation of donor tissue. Alternatively, certain compounds of the invention, or formulations thereof, may be appropriate for oral or enteral administration. Still other compounds of the invention will be suitable for topical administration.

In further embodiments, the CD40:CD154 binding interruptor is provided indirectly to the recipient, by administration of a vector or other expressible genetic material encoding the interruptor. The genetic material is internalized and expressed in cells or tissue of the recipient, thereby producing the interruptor in situ. For example, a suitable nucleic acid construct would comprise sequence encoding one or more of the MAb 5c8 immunoglobulin (Ig) chains as disclosed in U.S. Pat. 5,474,771. Other suitable constructs

would comprise sequences encoding chimeric or humanized versions of the MAb 5c8 Ig chains or antigen-binding fragments thereof. Still other suitable constructs would comprise sequences encoding part or all of other CD154-specific MAbs. The construct is delivered systemically or locally, e.g., to a site vicinal to the site of implantation of insulin-expressing tissue.

Alternatively, the vector or other genetic material encoding the interruptor is internalized within a suitable population of isolated cells to produce interuptor-producing host cells. These host cells then are implanted or infused into the recipient, either locally or systemically, to provide in situ production of the CD40:CD154 binding interruptor. Appropriate host cells include cultured cells, such as immortalized cells, as well as cells obtained from the recipient (e.g., peripheral blood or lymph node cells, such as natural killer (NK) cells).

Formulation

In general, the compound(s) used in practice of the invention are suspended, dissolved or dispersed in a pharmaceutically acceptable carrier or excipient. The resulting therapeutic composition does not adversely affect the recipient's homeostasis, particularly electrolyte balance. Thus, an exemplary carrier comprises normal physiologic saline (0.15M NaCl, pH 7.0 to 7.4). Another exemplary carrier comprises 50 mM sodium phosphate, 100 mM sodium chloride. Many other acceptable carriers are well known in the art and are described, for example, in Remington's Pharmaceutical Sciences, Gennaro, ed., Mack Publishing Co., 1990. Acceptable carriers can include biocompatible, inert or bioabsorbable salts, buffering agents, oligo- or polysaccharides, polymers, viscosity-improving agents, preservatives, and the like.

Any CD40:CD154 binding interruptor, such as a CD154 blocking agent, that is used in practice of the invention is formulated to deliver a pharmaceutically-effective or therapeutically-effective amount or dose, which is an amount sufficient to produce a detectable, preferably medically beneficial effect on the recipient. Medically beneficial effects would include preventing, delaying or attenuating deterioration of, or detectably

improving, the recipient's medical condition. As an example, the titer of an inhibitory antibody specific for a needed, exogenous protein therapeutic can be suppressed or lowered. Thus, for example, an effective amount of a therapeutic compound of the invention, such as a CD154 blocking agent, is any amount which detectably restores therapeutic efficacy of the protein therapeutic. An optimal effective amount is one which substantially frees the subject of counter-adaptive antibodies that give rise to the inhibitor syndrome.

Dosages and Frequency of Treatment

The amount of and frequency of dosing for any particular compound to be used in practice of the invention is within the skills and clinical judgement of ordinary practitioners of the medical arts, such as physicians. The general dosage and administration regime is established by preclinical and clinical trials, which involve extensive but routine studies to determine effective, e.g., optimal, administration parameters for the desired compound. Even after such recommendations are made, the practitioner will often vary these dosages for different subjects based on a variety of considerations, such as the subject's age, medical status, weight, sex, and concurrent treatment with other pharmaceuticals. Determining effective dosage and administration regime for each CD40:CD154 binding interruptor used in the invention is a routine matter for those of skill in the pharmaceutical and medical arts. The dosage amount and timecourse of should be sufficient to produce a clinically beneficial change in one or more indicia of the subject's health status. Exemplary timecourse and dosage regimes are set forth in the proof-of-principle studies included herein.

To exemplify dosing considerations for an anti-CD154 compound, the following examples of administration strategies are given for an anti-CD154 MAb. The dosing amounts could easily be adjusted for other types of CD154 blocker compounds. In general, single dosages of between about 0.05 and about 50 mg/kg subject body weight are contemplated, with dosages most frequently in the 1-20 mg/kg range. To initiate CD154 blockade therapy prophylactically, when the subject is in remission, or for emergency

therapy of acute disease, an effective dose of MAb ranges from about 1 mg/kg body weight to about 20 mg/kg body weight, administered daily or at intervals ranging from two to five days, for a period of about three weeks. Therapy can be maintained by administering the MAb intermittently thereafter, in dosages ranging from about 0.1 mg/kg body weight to about 20 mg/kg body weight. For maintenance purposes, the interdose interval may range from about one week up to about three months. At present, a one-month (four week) interdose interval is preferred.

CD154 blockade therapy can be practiced, if desired, serially or in combination with conventional immunosuppression therapy. A conventional immunosuppressant agent (e.g., a corticosteroid or calcineurin inhibitor) can be co-administered at any point during CD154 blockade therapy deemed prudent by the practitioner. Alternatively, a CD154 blocking MAb may be conjugated to a conventional agent. This advantageously permits the administration of the conventional agent in an amount less than the conventional dosage, for example, less than about 50% of the conventional dosage, when the agent is administered as monotherapy. Accordingly, the occurrence of many side effects associated with that agent should be avoided. Thus, according to this invention, CD154 blocking MAbs can be used together with other agents targeted at B cells, such as anti-CD19, anti-CD28 or anti-CD20 antibody (unconjugated or radiolabeled), IL-14 antagonists, LJP394 (LaJolla Pharmaceuticals receptor blocker), IR-1116 (Takeda small molecule) and anti-Ig idiotype monoclonal antibodies. Alternatively, the combinations may include T cell/B cell targeted agents, such as CTLA4Ig, IL-2 antagonists, IL-4 antagonists, IL-6 antagonists, receptor antagonists, anti-CD80/CD86 monoclonal antibodies, TNF, LFA1/ICAM antagonists, VLA4/VCAM antagonists, brequinar and IL-2 toxin conjugates (e.g., DAB), prednisone, anti-CD3 MAb (OKT3), mycophenolate mofetil (MMF), cyclophosphamide, and other immunosuppressants such as calcineurin signal blockers, including without limitation, tacrolimus (FK506). Combinations may also include T cell targeted agents, such as CD4 antagonists, CD2 antagonists and IL-12.

Pre-Clinical Model Systems for Evaluating CD40:CD154 Interruptor Treatment Regimes

Currently preferred, exemplary model systems for testing efficacy of a CD40:CD154 interrupting compound (e.g., an anti-CD40L compound or a CD154 blocking agent, such as a MAb having the specificity of MAb 5c8) are set forth below. In each system, routine modifications or adaptations can be made, to tailor the published techniques as needed to assess the effects of any desired CD40:CD154 interrupting compound on the status of protein inhibitory titers in the model animal. Some exemplary modifications are mentioned in the following brief summaries; however, many other appropriate modifications will be apparent to the skilled practitioner and are contempleted herein.

Knockout Mouse Model for Hemophilia A.

Recently, investigators at the American Red Cross have established a breeding colony of mice rendered nullizygous ("knocked out") for native murine FVIII. Bi et al. (1995), 10 Nature Genetics 119. These mice exhibit all relevant pathologies of human hemophilia A. Furthermore, the mouse model accurately mimics the etiology of these disease pathologies: hereditary or congenital absence of biologically active, native FVIII. Bolus administration of human FVIII, administered in a manner corresponding to conventional FVIII replacement therapy, has been reported to trigger the production of FVIII inhibitor antibodies in these mice. Quian et al. (1996), 88 Blood 656a (suppl.). Other routes of administration, specifically constitutive replacement via integration of an adenoviral vector encoding functional FVIII, appear currently to present the protein therapeutic in a less immunogenic context. Connely et al. (1998), 91 Blood 3273-3281.

The effects of a CD154 blocking agent, e.g., an anti-murine CD154 on the development of "high responders" in a population of the above-described hemophiliac mice can be assessed generally as follows: the antigen (FVIII) can be injected as a bolus dose (e.g., 0.2 ug) on study days 0 and 14. On or about study day 54, a blood sample can be withdrawn and assayed (using routine ELISA techniques) for presence of FVIII

inhibitory antibodies. Thereafter, a test group (e.g., 5 or more animals; a similar number of animals can be assigned to one or more appropriate control groups) can be provided with an appropriate dose of the anti-murine CD154 (e.g., 250 ug, i.p. or i.v.), for example on or about study days 55 and/or 57. A challenge dose of FVIII can be administered on or about study day 56. Thereafter, blood samples can be withdrawn and assayed on appropriate study days to monitor the development and, in the test group, suppression or reversal of a secondary response of inhibitor antibodies to FVIII. For example, bloods can be obtained at or about study days 74, 81 and 96. Allowing for some individual variation between animals in the test group, it is expected that CD154 blockade therapy will significantly blunt or suppress secondary humoral immunity to FVIII.

SCID-hu Chimeric Mouse Model.

This chimeric mouse model system, originally reported by Mosier et al. (1988), 335 Nature 256-259, is based on immunological rescue (functional reconstitution) of severe combined immunodeficiency (SCID) mice by engraftment of normal human peripheral blood leukocytes (PBLs), resulting in a stable mouse-human chimera. This system has been used for numerous investigations of the behavior and dynamic interactions of human lymphocytes in vivo. Significantly, this model system has been used to investigate the effects of CD40:CD154 interrupting agents on the response of normal human leukocytes to murine erythrocytes (used as a model antigen). Chen et al. (1995), 155 J. Immunol. 2833-2840. In this study, anti-CD40 and anti-CD154 MAbs were shown to downmodulate total human Ig production.

This model system allows assessment of the effects of an anti-human CD154, e.g., hu5c8, on human T cells in vivo, using any desired protein as a test antigen. In one appropriate modification, SCID-hu mouse chimeras can be created by engraftment of human PBLs from hemophiliac subjects, such as high responders. Of course, this approach can be taken with PBLs from any human affected by an exogenous protein inhibitor syndrome. In the case of SCID-hu mice made from a hemophiliac high responder, appropriate numbers (e.g., 2 to 5 or more) mice can be assigned to study groups as follows:

Group A (hu5c8 and FVIII); Group B (hu5c8 alone); Group C (FVIII alone); Group D (vehicle only); Group E (control Ig and FVIII); Group F (control Ig alone). The indicated test article and/or control is admixed with the hu PBLs at the time of engraftment, and is provided i.p. on or about study days 2 and/or 4. Kinetics of the ensuing FVIII inhibitor response can be monitored by standard techniques (ELISA) using bloods withdrawn at suitable intervals over a several week period. Treatment with hu5c8 is expected to blunt or abrogate secondary humoral immunity to FVIII.

Non-human Primate Models.

AVONEX (IFN β) model. Rhesus or cynomologus monkeys are assigned to appropriate study groups, e.g., two to four animals per group, as follow: Group 1 (control antigen (HAS); 50 ug/kg and hu5c8, 5; mg/kg), Group 2 (vehicle and hu5c8; 5 mg/kg), Group 3 (AVONEX; 50 ug/kg and hu5c8; 5 mg/kg), Group 4 (AVONEX; 50 ug/kg and hu5c8; 5 mg/kg), Group 5 (vehicle and hu5c8; 5 mg/kg). Groups 1, 2 and 3 receive hu5c8 commencing on study day 1 and approximately every second or third day thereafter. Groups 4 and 5 receive hu5c8 commencing on study day 17 and approximately every second or third day thereafter. All AVONEX groups receive AVONEX q.o.d. beginning on or about study day 3. The development and kinetics of AVONEX inhibitor antibodies are monitored using routine ELISA techniques. Clear differences are expected between the AVONEX groups treated or untreated with hu5c8. Specifically, pretreatment with hu5c8 is expected to substantially blunt or abrogate the development of AVONEX inhibitor antibodies. Delayed treatment with hu5c8 is expected to substantially suppress or reverse the development of secondary humoral immunity to AVONEX.

The above-described model can be routinely adapted to assess the effects of hu5c8 or another CD154 blocking agent on primary and/or secondary inhibitor responses to other model antigens, including exogenous protein therapeutics. For example, routine, appropriate modifications of the protocol and dose levels can be made to assess the behavior of FVIII or another clotting factor in primates provided with prophylactic or therapeutic regimens of CD154 blockade therapy.

Equivalents

The invention may be embodied in other specific forms without departing from the spirit or essential characteristics thereof. The foregoing embodiments are therefore to be considered in all respects illustrative of, rather than limiting on, the invention disclosed herein. Scope of the invention thus is indicated by the appended claims rather than by the foregoing description, and all changes which come within the meaning and range of equivalency of the claims are intended to be embraced therein.

What is claimed is:

1. A method of attenuating severity of exogenous protein inhibitor syndrome, comprising the step of administering an effective amount of a CD40:CD154 binding interruptor to a subject afflicted with, or at risk of, said syndrome.

2. A method of suppressing adverse effects of exogenous protein inhibitor syndrome, comprising the step of administering an effective amount of a CD40:CD154 binding interruptor to a subject afflicted with, or at risk of, said syndrome.

3. A method of preventing development of exogenous protein inhibitor syndrome, comprising the step of administering an effective amount of a CD40:CD154 binding interruptor to a subject afflicted with, or at risk of, said syndrome.

4. A method of delaying onset of exogenous protein inhibitor syndrome, comprising the step of administering an effective amount of a CD40:CD154 binding interruptor to a subject afflicted with, or at risk of, said syndrome.

5. A method of inhibiting development of exogenous protein inhibitor syndrome, comprising the step of administering an effective amount of a CD40:CD154 binding interruptor to a subject afflicted with, or at risk of, said syndrome.

6. A method of reversing exogenous protein inhibitor syndrome, comprising the step of administering an effective amount of a CD40:CD154 binding interruptor to a subject afflicted with, or at risk of, said syndrome.

7. A method of preserving therapeutic activity of an exogenous protein, comprising the step of administering an effective amount of a CD40:CD154 binding interruptor to a subject being treated with said exogenous protein.

8. A method of restoring therapeutic activity of an exogenous protein, comprising the step of administering an effective amount of a CD40:CD154 binding interruptor to a subject being treated with said exogenous protein, to which the subject has developed an immune response.

9. A method according to claim 1, 2, 3, 4, 5, 6, 7 or 8, wherein the CD40:CD154 binding interruptor is a CD154 (CD40L) blocking agent.

10. A method according to claim 9, wherein CD154 blocking agent is a monoclonal antibody.

11. A method according to claim 10, wherein the monoclonal antibody has the antigen-specific binding characteristics of the 5c8 antibody produced by ATCC Accession No. HB 10916.

12. A method according to claim 1, 2, 3, 4, 5, 6, 7 or 8, wherein the exogenous protein is administered to replace an endogenous, but defective protein.

13. A method according to claim 1, 2, 3, 4, 5, 6, 7 or 8, wherein the exogenous protein has substantially the same as primary structure as a corresponding, endogenous protein, and is produced from an isolated host cell harboring expressible, recombinant nucleic acid encoding said exogenous protein.

14. A method according to claim 1, 2, 3, 4, 5, 6, 7 or 8, wherein the exogenous protein is of bacterial origin.

15. A method according to claim 12, wherein the exogenous protein is a clotting factor.

16. A method according to claim 15, wherein the clotting factor is Factor VIII or Factor IX.

17. A method according to claim 13, wherein the exogenous protein is a growth hormone, wound healing factor, growth factor, cytokine, lymphokine, enzyme, clotting factor, or plasma component.

18. A method according to claim 14, wherein the exogenous protein is streptokinase.

19. A method according to claim 1, 2, 3, 4, 5, 6, 7 or 8, wherein the subject is human.

20. A method according to claim 19, wherein the human is a hemophiliac.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 98/12773

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 A61K39/395 A61K38/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	FOY T M ET AL: "In vivo CD40-gp39 interactions are essential for thymus-dependent humoral immunity. II. Prolonged suppression of the humoral immune response by an antibody to the ligand for CD40, gp39." JOURNAL OF EXPERIMENTAL MEDICINE, (1993 NOV 1) 178 (5) 1567-75. JOURNAL CODE: I2V. ISSN: 0022-1007., XP000647639 United States cited in the application see the whole document --- -/--	1-20

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
8 October 1998	23. 10. 98

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Mennessier, T

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Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 98/12773

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	MOHAN C ET AL: "Interaction between CD40 and its ligand gp39 in the development of murine lupus nephritis." JOURNAL OF IMMUNOLOGY 154 (3). 1995. 1470-1480. ISSN: 0022-1767, XP002062342 cited in the application see the whole document	1-20
A	DURIE, FIONA H. ET AL: "Prevention of collagen - induced arthritis with an antibody to gp39, the ligand for CD40" SCIENCE (WASHINGTON, D. C., 1883-) (1993), 261(5126), 1328-30 CODEN: SCIEAS;ISSN: 0036-8075, XP002064747 cited in the application see the whole document	1-20
A	NOELLE, RANDOLPH J.: "The role of gp39 (CD40L) in immunity" CLIN. IMMUNOL. IMMUNOPATHOL. (1995), 76(3, PT. 2), S203-S207 CODEN: CLIIAT;ISSN: 0090-1229, XP002080010 see the whole document	1-20
X	BUHLMANN J E ET AL: "Therapeutic potential for blockade of the CD40 ligand, gp39." JOURNAL OF CLINICAL IMMUNOLOGY, (1996 MAR) 16 (2) 83-9. REF: 56 JOURNAL CODE: HRC. ISSN: 0271-9142., XP002079820 United States see the whole document	1-20
E	WO 98 30241 A (BIOGEN INC ;KALLED SUSAN L (US); THOMAS DAVID W (US)) 16 July 1998 see page 2, line 28-29; claim 24	1-20

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Form PCT/ISA/210 (continuation of second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 98/12773

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Although claims 1-20 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the CD40:CD154 binding interruptor..

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a)

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

Internat...al Application No
PCT/US 98/12773

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9830241 A	16-07-1998	WO 9839026 A	11-09-1998